

# Isolation and characterization of BanLec-I, a mannoside-binding lectin from *Musa paradisiac* (banana)

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A lectin (BanLec-I) from banana (*Musa paradisiac*) with a binding specificity for oligomannosidic glycans of size classes higher than (Man)<sub>6</sub>GlcNAc was isolated and purified by affinity chromatography on a Sephadex G-75 column. It did not agglutinate untreated human or sheep erythrocytes, but it did agglutinate rabbit erythrocytes. BanLec-I stimulated T-cell proliferation. On size-exclusion chromatography, BanLec-I has a molecular mass of approx. 27 kDa, and on SDS/PAGE the molecular mass is approx. 13 kDa. The isoelectric point is 7.2–7.5. BanLec-I was found to be very effective as a probe in detecting glycoproteins, e.g. on nitrocellulose blots.

## INTRODUCTION

During an investigation of the human antibody response to various foods (Calkhoven *et al.*, 1991), we found a marked binding of IgG4 to banana (*Musa paradisiac*) extract. In view of the mucilaginous nature of the banana extract, we considered the possibility of non-specific binding to IgG4 and decided to purify the active principle. We found that this binding activity was largely removed by gel filtration over a Sephadex G-75 column and was recovered by elution with mannoside. This clearly indicated the presence of a lectin. We describe here the physico-chemical properties of this lectin, which we have named BanLec-I.

## MATERIALS AND METHODS

### Isolation and purification

Bananas were purchased from the local grocery store. After discarding the skin, 100 g of fruit was extracted in 1 litre of 250 mM-NaCl/5 mM-MgCl<sub>2</sub>/5 mM-MnCl<sub>2</sub>/5 mM-CaCl<sub>2</sub>. After blending, the pH of the extract medium was adjusted to 7.5 by dropwise addition of 4 M-NaOH. After 15 min,  $\alpha$ -methyl D-mannopyranoside (mannoside) was added to a final concentration of 0.1 M. The suspension was stirred for 1 h at room temperature and this solution was centrifuged for 30 min at 27500 g. The supernatant was defatted with Freon TF (33%, v/v) (Hoekloos, Amsterdam, The Netherlands) by mixing the two phases on a horizontal shaker for 1 h and again centrifuged for 0.5 h at 27500 g. NaN<sub>3</sub> was added to a final concentration of 0.1% to the defatted supernatant, which was then dialysed against water for approx. 100 h with two changes each day. After dialysis, the extract was equilibrated to phosphate-buffered saline (PBS), pH 7.4, by addition of 10 × concentrated PBS and passed through 200 ml of PBS-equilibrated Sephadex G-75 (Pharmacia, Uppsala, Sweden) per litre of extract. The Sephadex was then washed with 4 column vol. of PBS. The bound material was eluted with 0.5 M-mannoside in PBS. The eluted fractions (still containing the mannoside used for elution) were tested for their inhibition in the competitive radioimmunoassay (see below). The active fractions were pooled and concentrated to 10 ml by

ultrafiltration on an Amicon UM-10 filter. This concentrate was dialysed against PBS containing 0.1% NaN<sub>3</sub> for approx. 3 days and then for another 1 day against PBS. The extraction procedure was performed at room temperature, and the dialysis was performed at 4 °C.

### Protein assays

Protein assays were performed with the bicinchoninic acid reagent obtained from Pierce (Rockford, IL, U.S.A.), according to the 'enhanced' protocol at 60 °C, using BSA and human  $\gamma$ -globulin as reference proteins; no differences were found between results using the two reference proteins.

### Detection of BanLec-I

For detection we used a standard radioimmunoassay procedure, in which human antibodies are used in combination with Protein A bound to Sepharose 4B as IgG-precipitating reagent. Protein A–Sepharose 4B [Pharmacia; 50  $\mu$ g in 100  $\mu$ l of PBS containing 0.05% Tween-20, 0.1% NaN<sub>3</sub> and 0.1% human serum albumin (PBS-AT)] was preincubated with 50  $\mu$ l of 1:400-diluted pooled normal human serum for 3 h at room temperature. The Sepharose was washed twice with PBS containing 0.05% Tween-20 and 0.1% NaN<sub>3</sub> (PBS-T) and lastly with PBS-AT containing 100 mM-mannoside. To this was added 50  $\mu$ l of the unknown sample and 50  $\mu$ l of <sup>125</sup>I-labelled BanLec-I. After overnight incubation at room temperature, the Sepharose was washed and the bound radioactivity was measured. In the absence of an inhibitor, 61% binding of the labelled lectin was obtained; 50% inhibition was achieved with banana extract equivalent to 25  $\mu$ g of banana and 1 ng of purified BanLec-I. The background binding in the absence of serum was 0.3%. We included an excess (0.05 M final concentration) of mannoside in the assay buffer, because otherwise the lectin (the labelled lectin and/or the lectin in the sample) might form complexes with any glycoprotein that might be in the sample, and this complex formation could influence the reactivity of the lectin with the antibodies.

### Iodination

The optimal iodination pH was established using phosphate buffers from pH 7 to pH 9 at intervals of 0.5 pH units. Iodination

Abbreviations used: PBS, phosphate-buffered saline (10 mM-sodium phosphate/140 mM-NaCl, pH 7.4); PBS-T, PBS containing 0.05% Tween-20 and 0.1% NaN<sub>3</sub>; PBS-AT, PBS-T containing 0.1% human serum albumin; ConA, concanavalin A; PLA, phospholipase A.

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at pH 7 was found to be most effective, with 83 % incorporation, compared with 3 % incorporation at pH 9. A 200  $\mu$ l sample of BanLec-I (0.05  $A_{280}$  units, containing 35  $\mu$ g of protein) was mixed with 66  $\mu$ l of 250 mM-phosphate buffer, pH 7, and 1 mCi of carrier-free  $^{125}$ I (Amersham). The reaction was initiated with 20  $\mu$ g of chloramine-T and stopped after 1 min by addition of 50  $\mu$ g of sodium metabisulphite in 50  $\mu$ l of PBS. The iodinated material was passed over a Bio-Rad P-10 column (40 cm  $\times$  1.2 cm) equilibrated with PBS-AT. The column was eluted with 50 mM-phosphate buffer, pH 7. The iodinated lectin was pooled and stored at  $-30^{\circ}\text{C}$  in small portions until further use. Concanavalin A (ConA; 50  $\mu$ g) (Miles, Yeda, Israel; lot no. 215) in 200  $\mu$ l of PBS was iodinated in the same way and used fresh.

#### Hyperimmune rabbit antisera

Partially purified BanLec-I was prepared on a phenyl-Sepharose CL-4B (Pharmacia) by hydrophobic interaction chromatography of the banana polyvinylpyrrolidone extract (Bjorksten *et al.*, 1980) in 1 M-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The bound material that was eluted from the phenyl-Sepharose at 0.2 M-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was dialysed and used for immunization. A pre-immune serum sample was obtained from two New Zealand White rabbits. The rabbits were immunized with an 925  $\mu$ g of protein (containing 25  $\mu$ g of BanLec-I), emulsified with equal volume of complete Freund's adjuvant. After 4 weeks, the rabbits were boosted with the same antigen in incomplete Freund's adjuvant. A serum sample was obtained from both rabbits after 1 week, tested and confirmed for a specific antibody response to BanLec-I. On 3 alternative days, a plasmapheresis was obtained from these rabbits. The plasma was clotted by recalcification, pooled and dialysed against PBS.

Other hyperimmune antisera to major allergens Fel d I (cat), Lol p I (English rye grass pollen), Der p I (mites) and phospholipase A<sub>2</sub>; bee venom were prepared in our Institute.

#### Characterization of the BanLec-I molecule

Pollen, food and invertebrate extracts were coupled to CNBr-activated Sepharose (Aalberse *et al.*, 1981). These Sepharose-coupled glycoproteins were then incubated overnight with radio-labelled BanLec-I. On the following day the Sepharose was washed and the bound radioactivity was measured. The bound radioactivity was eluted by incubating the Sepharose with increasing concentrations of mannoside. The Sepharose was washed with 0.9 % NaCl containing 0.05 % (w/v) Tween-20 and the bound radioactivity was measured. The same procedure was used for radiolabelled ConA.

Pollen, food and invertebrate extracts were electrophoresed on 12.5 % polyacrylamide gels in the presence of SDS after reducing their disulphide bonds in the presence of 10 % (v/v) 2-mercaptoethanol in the sample buffer (Laemmli, 1970). On completion, the separated proteins were electroblotted (Towbin *et al.*, 1979) on to a nitrocellulose sheet (Schleicher and Schuell, type BA 85), after which the nitrocellulose sheet was cut into 5 mm strips for further testing. Each strip was washed three times with PBS containing 0.01 % (v/v) Tween and 0.01 % (w/v) human serum albumin and then incubated in the same buffer containing 40000 c.p.m. of  $^{125}$ I-BanLec-I or  $^{125}$ I-ConA.

#### Haemagglutination and T-cell proliferation

Human erythrocytes of blood types A, B and O, as well as erythrocytes of blood type O treated with bromelain, papain (Low, 1955) and neuraminidase (Judd *et al.*, 1979), were obtained from the Department of Immunohaematology of our Institute. Bromelain-treated erythrocytes were prepared by mixing 1 part of 0.5 % (w/v) bromelain with 9 parts of 4 % (v/v) erythrocyte

suspension in PBS. After incubating the mixture for 30 min at  $37^{\circ}\text{C}$  in a water bath, the erythrocytes were washed three times with 9 vol. of PBS (centrifugation at 1500  $g$  for 10 min at room temperature) and resuspended in a 3 % (v/v) suspension.

Direct agglutination tests were performed in glass tubes with 50  $\mu$ l of BanLec-I (10  $\mu$ g/ml) in PBS with 5 mg of polyvinylpyrrolidone K90/ml (Fluka AG, Buchs, Switzerland) which was mixed with an equal volume of a 3 % (v/v) suspension of erythrocytes (from rabbits, unless indicated otherwise). For inhibition tests, the lectin was first mixed with 50  $\mu$ l of inhibiting sugar (0.1–300 mM) and then a 50  $\mu$ l erythrocyte suspension was added. After mixing the contents, the tubes were centrifuged for 1 min at 120  $g$  and the agglutination was scored macroscopically. The sugars used for inhibition of agglutination were mannose, GlcNAc, galactose, GalNAc, lactose and fucose (Sigma, St. Louis, MO, U.S.A.);  $\alpha$ -methyl D-mannopyranoside and  $\alpha$ -methyl D-glucopyranoside (Fluka); fructose and maltose (Baker, Deventer, The Netherlands); ribose and xylose (Merck, Darmstadt, Germany) and N-acetylneuraminic acid (Koch-Light Laboratories, Colnbrook, Bucks., U.K.).

Purified peripheral blood T-cells (40000 per well) without monocytes were cultured in the presence or absence of interleukin-2 with serial dilutions of BanLec-I. After 4 days the cells were labelled with [ $^3\text{H}$ ]thymidine for 4 h. The cells were then harvested and the incorporated [ $^3\text{H}$ ]thymidine was measured (Roosnek *et al.*, 1985).

#### Standard oligosaccharides or glycopeptides

For the determination of the binding specificity of immobilized BanLec-I radioactively labelled glycopeptides or oligosaccharides were used which were characterized by  $^1\text{H}$ -n.m.r. or capillary gas chromatography/mass fragmentography, as previously described (Diabaté *et al.*, 1984; Bierhuizen *et al.*, 1988, 1989a,b; Bierhuizen, 1989). [ $^3\text{H}$ ]Man-labelled (Man)<sub>6</sub>GlcNAcOH {Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3[Man $\alpha$ 1 $\rightarrow$ 3-(Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 6)Man $\alpha$ 1 $\rightarrow$ 6]Man $\beta$ 1 $\rightarrow$ 4GlcNAcOH} and (Man)<sub>6</sub>GlcNAcOH {Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3[Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3(Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 6)Man $\alpha$ 1 $\rightarrow$ 6]Man $\beta$ 1 $\rightarrow$ GlcNAcOH} were kindly donated by Dr. R. Geyer (Biochemisches Institut am Klinikum der Universität Giessen, Giessen, Germany) (Diabaté *et al.*, 1984). The (Man)<sub>6</sub>-substituted glycopeptide AC-D3 {Man $\alpha$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3[Man $\alpha$ 1 $\rightarrow$ 3(Man $\alpha$ 1 $\rightarrow$ 6)Man $\alpha$ 1 $\rightarrow$ 6]Man $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ peptide} was prepared from ovalbumin, labelled with [ $^{14}\text{C}$ ]acetate in the peptide part and identified by  $^1\text{H}$ -n.m.r. spectroscopy, as previously described (Bierhuizen *et al.*, 1989a).

Asialo complex-type biantennary glycopeptide GP2 [Gal $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3(Gal $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 6)Man $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ Asn-Lys] and asialo complex-type triantennary glycopeptide MGP3 [Gal $\alpha$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 4(Gal $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 2)Man $\alpha$ 1 $\rightarrow$ 3(Gal $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 6)Man $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ Asn-Lys] were originally prepared from human  $\alpha_1$ -acid glycoprotein and labelled with [ $^{14}\text{C}$ ]acetate in the peptide part, as previously described (Bierhuizen *et al.*, 1988).

Core-fucosylated complex-type monoantennary glycopeptide MS {NeuAc $\alpha$ 2 $\rightarrow$ 6Gal $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 2Man $\alpha$ 1 $\rightarrow$ 3(Man $\alpha$ 1 $\rightarrow$ 6)Man $\beta$ 1 $\rightarrow$ 4GlcNAc $\beta$ 1 $\rightarrow$ 4(Fuc $\alpha$ 1 $\rightarrow$ 6)GlcNAc $\beta$ 1 $\rightarrow$ [acetate- $^{14}\text{C}$ ]peptide} was a gift from Dr. D. H. van den Eijnden (Department of Medical Chemistry, Free University, Amsterdam, The Netherlands). ConA-Sepharose-binding fraction C3 of human hepatocytes was prepared from the membrane fraction of [ $^3\text{H}$ ]mannose-labelled human hepatocytes after extensive treatment with Pronase and ConA-Sepharose chromatography of the resulting glycopeptide fraction (Bierhuizen, 1989). The strongly bound glycopeptides of fraction C3 co-eluted from the

ConA-Sepharose column with the standard oligosaccharides (Man)<sub>6</sub>GlcNAcOH and (Man)<sub>6</sub>GlcNAcOH.

### Characterization of binding specificity of BanLec-I

BanLec-I-agarose chromatography was performed using a 1 ml column (0.17 cm × 11.5 cm; 6 ml/h; 0.6 ml fractions; 20 °C), with 10 mM-Tris/HCl (pH 7.5)/0.1 M-NaCl/0.02 % NaN<sub>3</sub> as starting buffer. After application in 0.2 ml of starting buffer, the standard glycopeptides or oligosaccharides were allowed to interact with the lectin for 1 h. Elution was then started with 9 column vol. of starting buffer. Glycans bound to the lectin were eluted specifically with 0.15 M- $\alpha$ -methyl glucopyranoside in starting buffer. The BanLec-I-agarose column was subsequently regenerated with 10 column vol. of starting buffer.

### Molecular mass and pI determination

A Sephadex G-75 column was prepared (100 cm × 1.2 cm) with PBS containing 0.1 M-mannoside and 0.01 % human serum albumin. Radiolabelled BanLec-I was eluted by gel filtration from this column along with other radiolabelled proteins with known molecular masses. The following radiolabelled proteins were used as markers: Fel d I, 35 kDa (Chapman *et al.*, 1988); Lol p I, 32 kDa (Cottam *et al.*, 1986; Ford & Baldo, 1986); Der p I, 24 kDa (Chapman & Platts-Mills, 1980) and PLA<sub>2</sub>, 24 kDa (Shipolini *et al.*, 1974; Hoffman & Shipman, 1976).

SDS/PAGE for molecular mass determination was performed on a 12 % gel in the Phast system of Pharmacia; the proteins were reduced with 10 % 2-mercaptoethanol and electrophoresed. The above-mentioned radiolabelled proteins were used, along with non-radioactive low-molecular-mass markers (Pharmacia). Isoelectric focusing (pH 3–10) for pI determination of the BanLec-I molecule was also carried out on the Phast system from Pharmacia. The above-mentioned radioactive proteins were used, along with non-radioactive pI markers (pI range 3–10, Pharmacia).

Agar immunoelectrophoresis of BanLec-I was performed in 1.3 % agar in 50 mM-barbitone buffer, pH 8.6, for 55 min at 10 V/cm. After electrophoresis, 150  $\mu$ l of 1:2 diluted rabbit anti-(banana lectin) antiserum was allowed to diffuse overnight from a trough cut in the gel parallel to the direction of electrophoresis.

## RESULTS

We compared five extraction procedures (Table 1) for the isolation of the lectin (all extracts refer to dialysed extracts). Most activity was found in the extract prepared from 100 g of peeled banana extracted in 1000 ml (final volume) of a buffer containing 250 mM-NaCl, 5 mM each of MgCl<sub>2</sub>, MnCl<sub>2</sub>, CaCl<sub>2</sub> and 0.1 M-mannoside. The maximum amount of lectin extracted was 3.8 mg/100 g of banana.

The yield of the purification of the BanLec-I (Table 2) from the dialysed banana extract was 57 %. We were not able to measure accurately any possible loss of BanLec-I during dialysis, because the undialysed extract formed a precipitate that interfered with the test assay.

We tested the reactivity of the lectin with extracts of 140 vegetable, invertebrate and mammalian materials. These extracts were coupled to CNBr-activated Sepharose and incubated with <sup>125</sup>I-labelled BanLec-I. The maximum binding of <sup>125</sup>I-BanLec-I to a glycoprotein was approx. 80 %. Some examples are shown in Fig. 1. Iodinated BanLec-I was considerably more reactive in these tests than <sup>125</sup>I-labelled ConA. When the Sepharose-bound lectin was incubated with 125 mM-mannoside, a larger fraction of BanLec-I than of ConA was eluted.

We also tested the binding of the two labelled lectins to

**Table 1. Yield of BanLec-I using five different procedures**

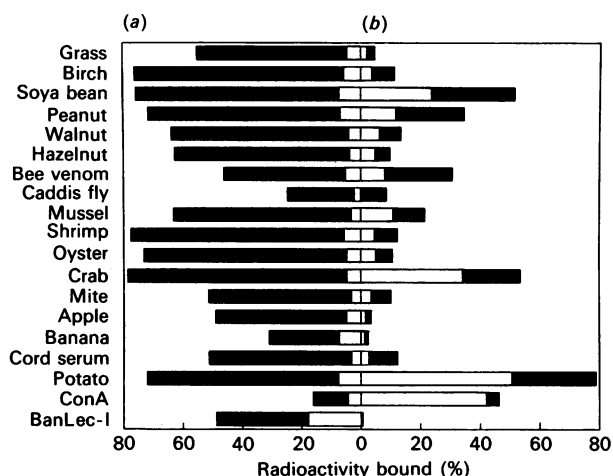
The results are expressed per 100 g of peeled banana. Medium A contained 250 mM-NaCl and 5 mM each of MnCl<sub>2</sub>, MgCl<sub>2</sub> and CaCl<sub>2</sub>. \*Value near lower limit of detection.

Extraction medium	Protein (mg/100 g)	BanLec-I activity	
		(mg/100 g)	( $\mu$ g/mg of protein)
Water extract	60	0.08	1.3
Water + 0.1 M-mannoside	150	0.12	0.8
Banana polyvinylpyrrolidone extract	79	0.18	2.2
Medium A	15*	2.0	134
Medium A in 0.1 M-mannoside	24*	3.8	170

**Table 2. Yield from affinity chromatography purification of BanLec-I on Sephadex G-75**

The results are expressed per 100 g of peeled banana. \*Value near lower limit of detection.

Stage of extraction	Protein (mg/100 g)	BanLec-I activity	
		(mg/100 g)	( $\mu$ g/mg of protein)
Dialysed extract	24*	3.8	170
Sephadex G-75 depleted	23*	0.05	3
BanLec-I	2.2	2.2	1000



**Fig. 1. Comparison of the binding of <sup>125</sup>I-BanLec-I and <sup>125</sup>I-ConA**

The extracts shown containing glycoproteins were coupled to CNBr-activated Sepharose 4B. The non-solubilized glycoproteins were then incubated with radiolabelled BanLec-I (a) or ConA (b). ■, Binding in the absence of mannoside; □, residual lectin binding after elution with 125 mM-mannoside. Negative control adsorbents were Protein A-Sepharose and glycine-inactivated CNBr-Sepharose. In the absence of mannoside, < 3 % of the radioactivity was bound.

glycoproteins blotted on to nitrocellulose (Fig. 2). Here again, BanLec-I was clearly superior. Qualitatively, almost identical patterns were obtained for BanLec-I and ConA, with a few exceptions, e.g. components in bee venom and hazelnut.

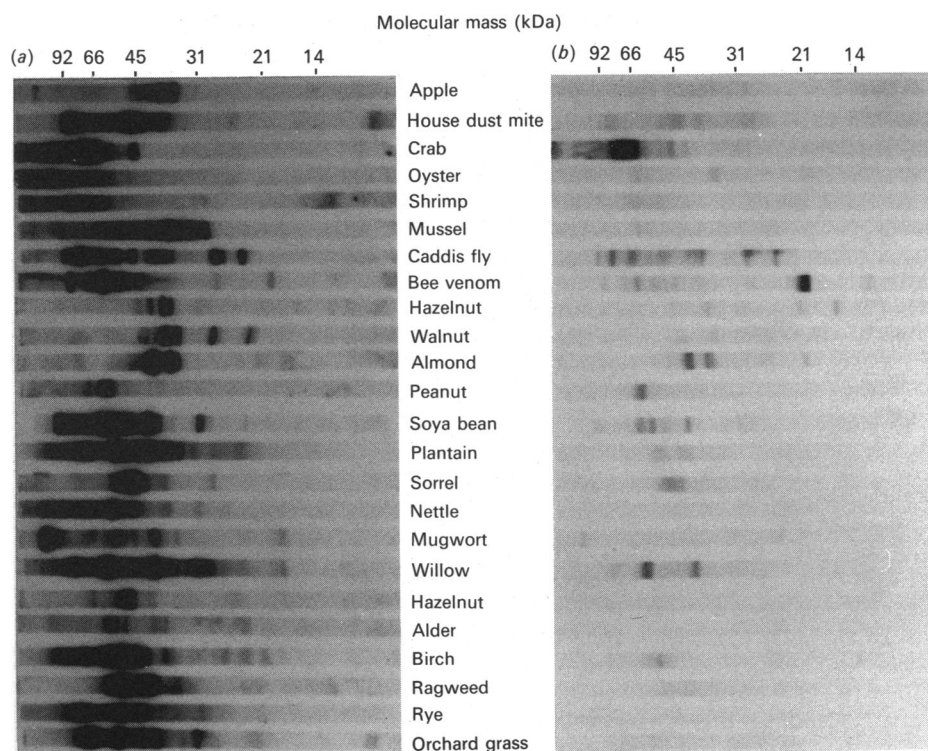


Fig. 2. Binding of BanLec-I and ConA to glycoproteins

Glycoprotein-containing extracts were reduced in the presence of 2-mercaptoethanol and electrophoresed on 12.5% polyacrylamide gels in the presence of SDS and electroblotted on to a nitrocellulose sheet. Strips (5 mm) of nitrocellulose sheet were incubated with radiolabelled BanLec-I (a) or ConA (b), washed, dried and exposed to a Kodak X-ray film for 36 h with an intensifying screen. The samples from plantain to orchard grass consisted of pollen.

### Biological activity of BanLec-I

Agglutination with BanLec-I of untreated erythrocytes was negative with human and sheep cells, but distinctly positive with rabbit cells, particularly in polyvinylpyrrolidone-containing buffer. Enzyme treatment of human erythrocytes resulted in weak lectin-induced agglutination in the case of bromelain, but not with papain or neuraminidase. The agglutination of the rabbit cells was inhibited by  $\alpha$ -methyl mannoside (end-point of inhibition at 0.4 mM), mannose (1.1 mM) and glucose (37 mM), but not by galactose, GalNAc, GlcNAc, lactose, fucose, ribose or xylose (all seven sugars tested at 300 mM).

BanLec-I stimulated T-cell proliferation. When purified, peripheral blood T-cells without monocytes were stimulated by BanLec-I in the presence of interleukin-2. Table 3 shows the proliferative response of the T-cells to serial dilutions of BanLec-I. Similar results were obtained with another batch of BanLec-I in another donor: i.e. no stimulation in the absence of interleukin-2, and a 25-fold stimulation by 900 ng of BanLec-I/ml in the presence of interleukin-2.

The binding specificity of BanLec-I was investigated using agarose-immobilized lectin. In Table 4 the elution characteristics are shown for a variety of standard complex-type and oligomannosidic glycans, which, with the exception of GP3, were specifically retarded on or bound to ConA-Sepharose (Bierhuizen *et al.*, 1989b). No binding or retardation was obtained for the six-mannose-residue-containing glycopeptide AC-D3, or for the various complex-type standard glycopeptides. Specific binding and elution of standard structures was obtained with the oligosaccharides (Man)<sub>6</sub>GlcNAcOH and (Man)<sub>6</sub>GlcNAcOH. Glycopeptides from human hepatocytes that co-eluted with the latter oligosaccharides from ConA-Sepharose were also retained specifically by BanLec-I-agarose.

Table 3. Proliferative response of purified human T-cells to BanLec-I

Results are expressed as c.p.m. of [<sup>3</sup>H]thymidine incorporated by 40 000 T-cells (counting efficiency 40%). The results represent the medians of triplicate assays. In the experiments with interleukin-2, its concentration in the medium was 50 units/ml. The results with 300, 1200 and 1800 ng of BanLec-I/ml were obtained with T-cells from a different donor and with a different lectin batch than the other results.

BanLec-I (ng/ml)	[ <sup>3</sup> H]Thymidine incorporation (c.p.m.)	
	– Interleukin-2	+ Interleukin-2
Donor A		
0	10	1800
56	30	1900
225	30	5000
900	100	9000
3600	200	10 500
Donor B		
0	20	600
300	50	15 500
1200	20	8900
4800	60	1300

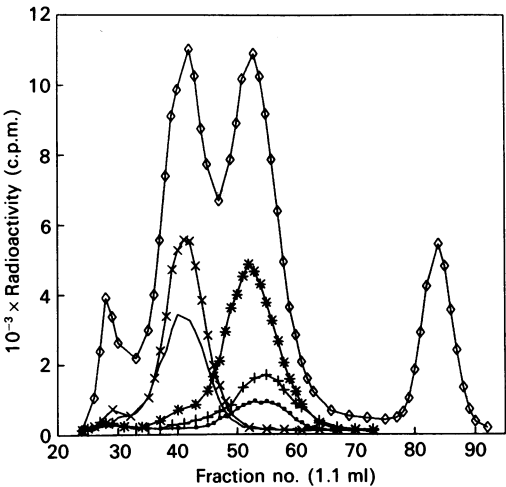
### Physicochemical characteristics

When size-exclusion chromatography of a mixture of <sup>125</sup>I-labelled BanLec-I and four iodinated marker proteins of known molecular mass was performed on Sephadex G-75 in the presence of 0.1 M-mannoside, BanLec-I was found to have a molecular mass of approx. 27 kDa (Fig. 3). On SDS/PAGE in the presence

**Table 4. Characterization of carbohydrate-binding specificity of BanLec-I agarose**

Glycans were eluted specifically with one column vol. of 0.15 M- $\alpha$ -methyl glucopyranoside. 'Not-retarded' glycans were eluted with one column vol. of starting buffer. See the Materials and methods section for details of the chromatographic procedure used and for the definition of the standard structures and of the ConA-Sepharose-binding fraction C3 of human hepatocytes.

Glycan	Binding characteristics
Asialo complex-type biantennary glycopeptide GP2	Not retarded
Asialo complex-type triantennary glycopeptide GP3	Not retarded
Core-fucosylated complex-type monoantennary glycopeptide MS	Not retarded
(Man) <sub>6</sub> -substituted glycopeptide AC-D3	Not retarded
(Man) <sub>6</sub> GlcNAcOH	Eluted specifically
(Man) <sub>6</sub> GlcNAcOH	Eluted specifically
ConA-Sepharose-binding fraction C3 of membrane-bound glycopeptides of human hepatocytes	Eluted specifically

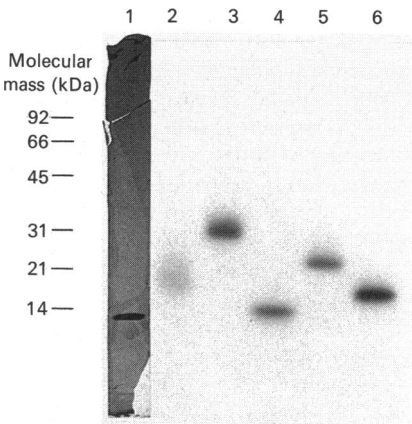


**Fig. 3. Molecular mass determination of <sup>125</sup>I-BanLec-I**  
This was carried out by gel filtration on Sephadex G-75 with radiolabelled proteins of known molecular mass Fel d I, 35 kDa (×); Lol p I, 32 kDa (–); Der p I (24 kDa; +); PLA, 24 kDa (●). \*, BanLec-I; ◇, total c.p.m. The buffer used for elution was PBS-AT containing 0.1 %  $\alpha$ -methylmannoside. Protein A was incubated with individual hyperimmune rabbit antiserum and tested with eluted fractions.

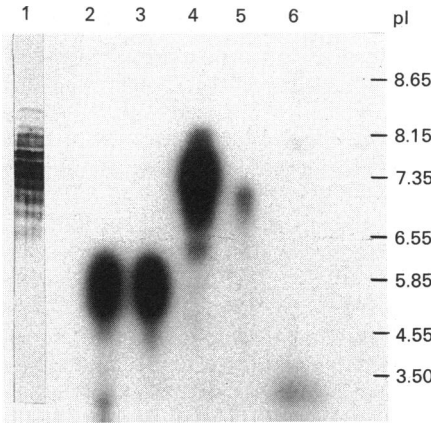
of a reducing agent (Fig. 4), a homogeneous band with a molecular mass of approx. 13 kDa was obtained. On isoelectric focusing (Fig. 5), we detected about 12 protein bands in the pI range 6.55–8.15. Most staining was found at pI 7.3. The results in agar immunoelectrophoresis at pH 8.6 were in agreement with this pI. BanLec-I produced a single precipitin line with B-electrophoretic mobility.

DISCUSSION

Banana contains a lectin which binds to molecules containing  $\alpha$ -D-mannopyranosyl and other, presumably related, carbohydrate structures. We propose its name to be BanLec-I. It is present in relatively low quantities: 0.04 mg of lectin/g of fruit



**Fig. 4. Autoradiograph of molecular mass determination by SDS/PAGE**  
<sup>125</sup>I-labelled protein samples were reduced in the presence of 2-mercaptoethanol and electrophoresed on the Phast system on a 12 % polyacrylamide gel. Low-molecular-mass markers (Pharmacia) are denoted on the left. Lane 1 is the BanLec-I, and lanes 2–6 are radiolabelled Fel d I (18 kDa), Lol p I (32 kDa), BanLec-I (13 kDa), Der p I (24 kDa), and PLA (17 kDa).



**Fig. 5. Autoradiograph of pI determination by isoelectric focusing**  
<sup>125</sup>I-labelled proteins were separated on the Phast system. Isoelectric focusing markers (Pharmacia) in the range pI 3–10 are denoted on the right. Lane 1 contains BanLec-I, and lanes 2–6 contain radiolabelled Fel d I, Lol p I, BanLec-I, Der p I and PLA respectively.

(59 % water), or 0.07 mg/g of dried fruit. This is low compared with the lectin content of jack beans: 26 mg of ConA/g of dried beans (Agrawal & Goldstein, 1972).

We considered the possibility that the lectin might be concentrated in the seeds. However, on a weight basis, a banana seed extract was no more potent than an extract of seedless flesh. We have not looked into the possibility that the amount of BanLec-I in the fruit itself might vary according to various ripening stages of the fruit.

Extracts prepared in the presence of mannoside were almost twice as potent as extracts prepared in the absence of mannoside. The most likely explanation is that the banana fruit itself contains insoluble carbohydrate structures that bind the lectin.

Krupe (1956) did not find agglutinating activity in extract of banana. In a few preliminary experiments, we too failed to find any haemagglutinating activity of purified BanLec-I with human erythrocytes of different blood groups and with sheep erythrocytes, but a distinct agglutinating activity was obtained towards rabbit erythrocytes which was mannoside-inhibitable. The lectin did, however, induce proliferation of human T-cells comparable

with that produced by ConA. This indicates that the lectin is most probably a bi- or poly-valent molecule.

Our binding studies indicate that BanLec-I is a mannose-specific lectin which is highly specific for oligomannosidic glycans containing 8 or 9 Man residues. In contrast with ConA, the lectin did not bind to mono- or bi-antennary complex-type glycopeptides, or to the Man<sub>8</sub> glycopeptide AC-D3. It does not seem likely that the lack of binding of the latter structures was due to their glycopeptide nature (as opposed to pure oligosaccharides), because a glycopeptide (fraction C3 from human hepatocytes) was bound by BanLec-I.

We observed inhibition of agglutination by the polyfucose fucoidan (results not shown). This suggested the possibility that the lectin has an affinity for 1→6Fuc residues linked to the internal GlcNAc residue of the core. However, the lack of binding of the core-fucosylated complex-type monoantennary glycoprotein MS makes this unlikely.

In many aspects BanLec-I seems very similar to other well-established lectins, but two properties deserve special mention. First, it is highly immunogenic in man, as indicated by the strong IgG4 antibody response. We have a clear evidence that the binding of the lectin to IgG4 antibodies in the presence of mannoside is a classical antigen-antibody reaction (V. L. Koshte, M. Aalbers, P. G. Calkhoven & R. C. Aalberse, unpublished work). This marked immunogenicity provided us with the tool for the quantitative assay of the lectin. Secondly, the lectin was found to be an excellent reagent for the detection of glycoproteins. It was found to be easily iodinated, and the iodinated material stored at -20 °C retained its activity over a period of several months. With <sup>125</sup>I-labelled ConA under the same conditions we found a rapid loss of activity.

Qualitatively, the binding specificities of BanLec-I and ConA were very similar, as illustrated by the results from the SDS/PAGE blots of various extracts, with a few notable exceptions such as bee venom and hazelnut. Quantitatively, however, major differences were found. The Sepharose-bound glycoproteins bound a larger fraction of the iodinated BanLec-I than of ConA. It is unlikely that this difference is solely due to a difference in specific activity. The molar quantities of the two lectins that were used for labelling were similar. The labelling efficiency of the BanLec-I was higher, but even after correction for the resulting difference in specific activity, the binding activity of BanLec-I was still higher. It is of interest that BanLec-I not only binds more avidly to glycoproteins, but that it is also more easily eluted by mannoside. The probable explanation for this is that ConA is less stable; it tends to form aggregates, and in doing so loses its sugar-binding activity.

We conclude that BanLec-I is an excellent tool for glycoprotein research. In qualitative as well as quantitative assays the iodinated lectin has proved to be a consistent and reproducible reagent with a relatively long shelf life.

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